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Extraction, Phytochemical Screening, Isolation and Identification of Bioactive Compounds from Extract of the plant Euphorbia Thymifolia Linn

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Abstract:

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defense mechanisms against predator such as microorganism, insects and herbivores which have been proved to be potentially active compounds. Euphorbia Thymifolia Linn (E. Thymifolia) is commonly known as 'duddi' or in Sanskrit means Laghu didhika or Raktavindaka. It belongs to the family Euphorbiceae. This plant is bitter, acrid, sweet and used as thermogenic, laxative and diuretic. This plant is widely used in the ayurveda to cure many diseases like vitiated condition of constipation, helminthiasis and ringworm skin diseases and leprosy. The aim of the present study is to examine E.Thymifolia Linn whole plant for phytochemical profile. ***Isolation*** *and* ***Identification*** *of bioactive compounds. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Isolation and characterization of bioactive compound from methanolic extract of E. Thymifolia has been conducted. The bioactive compound from methanolic extracts was isolated by several processes, such as TLC, column chromatography and preparative TLC. The isolated bioactive compound is identified by UV-Vis spectrophotometer, FT-IR, ^{1}H, 13C-NMR and Mass. The obtained compound is continued to the preparative TLC using chloroform: methanol (50:50, v/v) as eluent. The UV-Vis spectrum showed one peaks of maximum absorbance at 312.8nm. Then, the FT-IR spectrum showed several peaks that confirmed the presence of functional group of derivative of compound, i.e. 669.05, 928.58, 1070.85, 1215.51 and 1710.07cm-1. ^{1}H and 13C-NMR spectrum confirmed the bioactive compound present in plant. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.*

Keywords: Euphorbia Thymifolia, Qualitative phytochemical, Isolation, Bioactive compounds

Introduction:

Ayurveda stresses on the use of vegetable drugs. Plants are being used as medicine since ancient Times1,2. A numbers of bioactive compounds in medicinal plants, such as alkaloids, tannins, flavonoids, sterols, triterpenes, etc., are noted to play major role in

physiology and management of diseases3. Flavonoids constitute one of the most exclusive classes of compounds in medicinal plants4. The foremost important task in this paradigm is the screening of flavonoids in plants. Phenolic compound are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom, even if the type of compound present varies according to the phylum under consideration. Phenolic are uncommon in varies bacteris, fungi and algae. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenol is found5. Phenolic substances or polyphenols contain numerous varieties of compounds: flavonoids, phenolic acids, complex flavonoid and colored anthocyanins. These phenolic compounds are usually related to defense responses in the plant. However, phenolic metabolites play an important part in other processes, for instance incorporating attractive substance to accelerate pollination, coloring for camouflage and defense against herbivores, as well as antibacterial and atifungal activities6. Chromatographic studies of these compounds serves to be a very useful and reliable source in the process of bioactive compounds screening in plants7. According to the ethnobotanical information, it has been reported that the plant E. Thymifolia (family- Euphorbiaceae) is a medicinal herb used traditionally in dysentery, bleeding piles, gonorrhoea, dysmenorrhoea, amenorrhoea, helminthiasis, ringworm, chronic cough, asthma, bronchitis, cardiac debility, greying of hairs, skin diseases etc8, 9. E. Thymifolia have numerous pharmacological activities including antibacterial10, 11, antifungal12, antimicrobial13, anti inflammatory14,15, antiviral16, antispasmodic, bronchodilator, antibronchial, hypoglycaemic20-22, anticancer23,24 and antioxidant25. A number of chemical constituents are present in E. Thymifolia whole herb and its different parts. According to the literature, the whole plant contains epitaraxerol, n-hexacosanol, euphorbol, 24 methylene cycloartenol, 12-deoxy-4P-hydroxyphorbol-13 dodecanoate-20-acetate, 12-deoxy-4P-hydroxyphorbol-13 phenylacetate-20-acetate, 12-deoxyphorbol-13,2O-diacetate, quercetin-3P-galactoside, 12-deoxyphorbol-l3,20-diacetate, 12-deoxy-4Phydroxyphorbol-13-dodecanoate-20-acetate, n hexacosanol, esters, n-alkanes and sterols26,27. Roots contain taraxerol and triucallol. Leaves and stems consist of 4 trihydroxy flavone-7- glycoside26. contain ellagitannin dimers, euphorbins G and H with 12 known polyphenols27-32. Therefore, the aim of this research is extraction, isolation, phytochemical screening and identification of bioactive compounds from methanolic extract of E. Thymifolia whole herb through several processes, such as thin layer chromatography, column chromatography and preparative TLC. To identify the

structure of bioactive compound, several analyses has been conducted such as UV-Vis, FT-IR, 1H, 13C-NMR and mass.

Recently, advanced techniques have become available to reduce the loss of bioactive compound without increasing the extraction time. Therefore, microwave-assisted extraction is demonstrated to be a good technique in multiple fields, especially in the medicinal plant area. Moreover, this technique reduced the losses of the biochemical compounds being extracted Microwave-assisted extraction (MAE) has been used as an alternative to conventional techniques for the extraction of antioxidants because of its ability to reduce both time and extraction solvent volume In fact, the main objective of using MAE is to heat the solvent and extract antioxidants from plants with a lesser amount of these solvents.

Li et al. reported that conventional methods using various solvents presented less antioxidant activity and phenolic content than MAE [16]. Therefore, the finding confirmed that MAE was more effective at increasing antioxidant activity by measuring ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and total phenolic content (TPC). The efficiency of the microwave extraction can be changed through some factors such as extraction temperature, solvent composition, and extraction time. The extraction temperature was usually studied more than other factors due to its ability to increase the efficiency of the microwave extraction. Tsubaki et al. reported that 170 °C was the most effective temperature for extracting phenolic compounds from Chinese tea. In addition, increasing the extraction temperature beyond this point resulted in a reduced extraction yield [17]. Recently, Christophere et al. used a new microwave-assisted extraction (MAE) process, which converts energy to heat, thereby cooperating with solvents to extract a specific compound. Williams et al. showed many advantages of MAE, including lower solvent consumption,

Ultrasound-assisted extraction (UAE) has been used in diverse applications of food-processing technology to extract bioactive compounds from plant materials Ultrasound, with levels greater than 20 kHz, is used to disrupt plant cell walls, which helps improve the solvent's ability to penetrate the cells and obtain a higher extraction yield. UAE can use a low operating temperature through processing, maintaining a high extract quality for compounds. UAE is known to be one of the easiest extraction techniques because it uses common laboratory equipment such as an ultrasonic bath. In this technique, a smashed sample is mixed with the suitable solvent and placed into the ultrasonic bath, while temperature and extraction time are controlled UAE of various organic and inorganic samples can use a wide range of solvents. Common equipment used in ultrasound-assisted extraction includes an ultrasonic bath and an ultrasonic probe system.

Unfortunately, ultrasonic probe has two main negative properties mainly related to experimental repeatability and reproducibility

Tabarak et al. noted that green technology is necessary to protect the environment from toxic substances, Therefore, extraction of phenolic compounds by ultrasound has grown during recent years due to its role in reducing the amount of solvent and energy used. Corrales et al. have shown that UAE can break down plant tissue and work properly during the production process and release of active compounds in solvents with a high efficiency Results showed an increase in antioxidant activity from 187.13 μmol TE g−1DM to 308 μmol TE g−1DM by using UAE as an effective method to extract antioxidants from different sources. Recently, Albu et al. studied and applied the use of ultrasound to extract phenolic compounds from rosemary [2]. Multiple criteria have been compared including ultrasonic bath extractions, ultrasonic probe system, a shaking water bath at various temperatures, and different solvents to select the most efficient method. In all situations, the operation time was dramatically decreased by applying and using the ultrasonic bath and probe systems.

Similar behaviour was reported by Cho et al. when extracting resveratrol from grapes [2]. In another study, Barbero et al. suggested the use of ultrasound in different industries because of its positive effects in the extraction of capacious of hot peppers Moreover, the ultrasonic method had the ability to decrease the degradation of phenolics. Multinacci et al. compared the extraction time of phenolic compounds from strawberries with other extraction methods such as solid–liquid, subcritical water, and microwave-assisted method. The results confirmed that UAE was the most effective method.

Material and Method:

Plant material the whole plant material of E. Thymifolia was collected from the Betwa riverine zone of Vidisha during rainy seasons of the year 2014 and washed thoroughly with distilled water. It was identified and authenticated by Taxonomist Dr. Sunil Dubey, Department of Botany, St. Mary P. G. College, Vidisha (M.P.). A voucher specimen was procured which was deposited in Department of Botany and Microbiology, St. Mary's P. G. College, Vidisha (M. P.) for future reference and the specimen voucher no. of E. Thymifolia is 104105. Chemical reagents All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Extraction Procedure Defatting of plant material Journal of Drug Delivery & Therapeutics. 2019; 9(3):107-113 asthmatic17-19, methanolic extracts of E. Thymifolia. The above phytoconstituents

were tested as per the standard method37. Fractionation of the extract by thin layer and column chromatography Thin layer chromatography Leaves The presence of different phytoconstituents in the methanolic extract of plant E. Thymifolia was established by TLC on silica coated alumina plates G' 60 F254 plate of 0.2 mm thickness by using different solvent systems as mobile phase. The selective solvent system used for the phytoconstituents detection was Chloroform (50): Methanol (50). A spotting capillary was used to add the extract to the plate. To spot the plate, simply touched the end of the capillary tube to the coated side of the plate and placed the TLC plate in the development chamber. The bottle was filled with a small amount of the mobile phase of solvent system and capped with a cork till complete development. After the complete development of spots on TLC plates, UV Chamber under the wavelength 254 nm of UV light was used to visualize the spots and the spots color were noticed. Besides this, Iodine chamber and visible light was also used to visualize the spots. silica gel was used as a stationary phase which was poured in to the column then filled chloroform solvent as mobile phase and was run two three times for maintaining its flow by removing air bubbles entered during packaging of silica. Then loaded sample on the top of the column and filled the column with appropriate solvent systems. The selective solvent system used for phyto constituents detection was Chloroform: Methanol (50:50). Then, obtained purified fractions were separated on basis of their color characterization and were kept in separate vials for further analysis. Isolation and structural elucidation of the compound Plant material of E. Thymifolia was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with n-Hexane using soxhlation method. The extraction was continued till the defatting of the material had taken place. Extraction 100gm of dried plant material were exhaustively extracted with different solvent in increasing order of polarity i.e. n Hexane, petroleum ether, benzene, acetone, methanol and distilled water using Soxhlet apparatus for 4 days. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. Dried extract was collected in an air tight container and stored at 4°C for further analysis33-36. Qualitative analysis of phytochemicals The biologically active purified fractions of the extract were sent to PBRL (Pinnacle Biomedical Research Laboratory), Bhopal for spectral analysis (UV) and to the SAIF CDRI, Lucknow for spectral analysis (IR, HNMR, CNMR and Mass) and spectral graphs were obtained. Which were interpreted with the literature available in books of spectroscopic analysis39,40.

Results and Discussion:

The crude extracts so obtained after the maceration process, extracts were further concentrated on water bath

for evaporate the solvents completely to obtain the actual yield of extraction. Table 1: % Yield of plant material S. No. Solvents 1 N- hexane Euphorbia Thymifolia 3.65% 2. Pet. Ether 3. 1.56% Benzene The methanolic extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids.

Table 1: % Yield of plant material

S. No.	Solvents	*Euphorbia Thymifolia*
1	N- hexane	3.65%
2.	Pet. Ether	1.56%
3.	Benzene	2.96%
4.	Methanol	7.34%
5.	Aqueous	4.38%
6.	Acetone	3.83%

To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

A small portion of the dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. The outcomes of the results are discussed separately in the table 2

Table 2: Preliminary phytochemical screening of *Euphorbia Thymifolia* (Linn.) extracts.

S. No.	Tests	Phytochemicals	Results
1	• Mayer's test • Wagner's test • Dragendorff's test • Hager's test	Alkaloids	+
2	• Molisch's test • Benedict's test • Fehling's test	Carbohydrates	+
3	• Modified Borntrager's test • Legal's test	Glycosides	+
4	• Froth/Foam test	Saponins	–
5	• Salkowski's test • Libermann-Burchard test	Steroids	+
6	Gelatin test	Tannins	+
7	Alkaline Reagent test • Lead acetate test	Flavonoids	+
8	• Xanthoproteic test • Ninhydrin test	Proteins and amino acids	–
9	Test for Triterpenes	Triterpenes	+

(present), - (Absent)

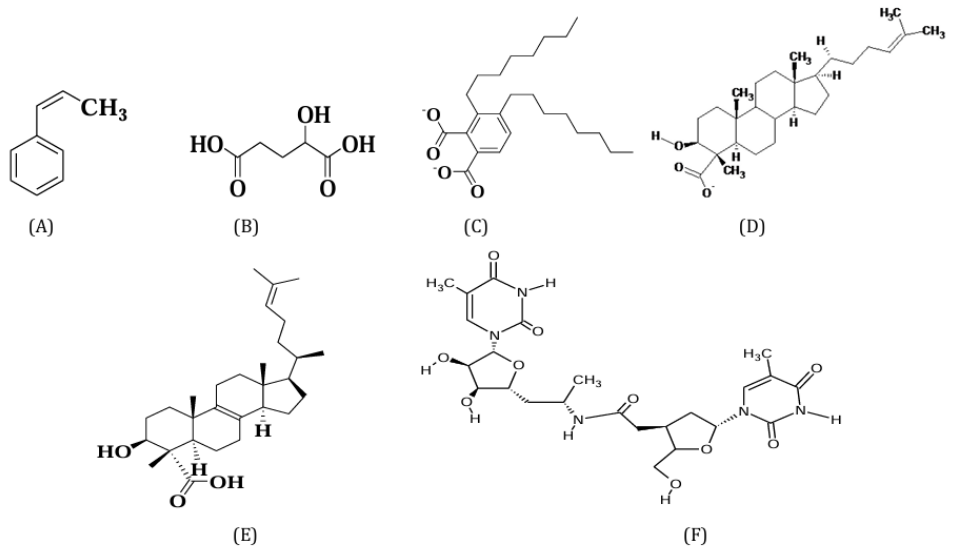

Figure 6: (A) Phenyl Propene (B) 2–Hydroxypentanedioic acid (C) Di-N-Octylphthalat (D) 2-methoxyisoxazolidine-3, 3, 5, 5-tetracarboxylic (E) 4alpha-Carboxy-4beta-methyl-5alpha-cholesta-8,24-dien-3beta-ol (F) 2-(5'- O-Methyl-3'-Deoxythymidin-3'-Yl)-N-[(S)-1(2'Alpha-Methoxy-3'-O-Methyl-5'-Deoxythymidin-5'-Yl) Ethyl]Acetamide

Conclusions:

From the result obtained it can be concluded that the whole plant of E. Thymifolia has medicinal values since it contains more secondary metabolites. Bioactive compound (Total 6) were isolated and identified by column, thin layer chromatography and which were subjected to spectral analysis i.e. IR, UV, 1D NMR (1H-NMR and 13C-NMR) and mass spectroscopy from methanol extract of E. Thymifolia (ET-2) whole herb. These compounds have been reported for the first time in this plant and can serve as a useful tool in its standardization. Methanolic extracts shows good results regarding presence of phytoconstituents hence these plants may directly use in medicine preparation or for the development of novel agents for various pathological disorders. Further research on the health benefits of phytochemicals in this plant may be warranted. Conflict of interest 8. 9. Mitra R. Bibliography on Pharmacognosy of Medicinal Plants, National Botanical Research Institute, Lucknow, 1985; 239. Siddiqui MB, Husain W. Traditional treatment of gonorrhoea through herbal drugs in the province of Central Uttar Pradesh, India. Fitoterapia 1993; 64(5):399-403. 10. Khan NH, Rahman M, Nur-e-Kamal MSA. Antibacterial activity of Euphorbia thymifolia Linn. Indian J Med Res 1988;87(6):395-7. 11. Banerjee DK, Pal DC. Plants used by the tribal of plain land in India for hair and scalp preparation, Fourth Int. Congr. Ethnobiol., NBRI, Lucknow 1994; 17(21):340. 12. Pal S, Gupta I. In vitro studies on the antifungal activity of Chotidudhi plant (Euphorbia prostrata Ait. and Euphorbia thymifolia Linn.). Indian J Pharmacol 1971; 3:29. The authors declare no conflict of interest.

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